## REMARKS

The issues outstanding in the Office Action mailed January 22, 2008, are the sequence rules compliance, the objections to the claims and the new rejections under 35 U.S.C. 102 and 103. Reconsideration of these issues, in view of the following discussion, is respectfully requested.

At the outset, the Examiner is thanked for indicating withdrawal of the majority of issues in the prior Office Action. It is submitted that entry of the foregoing amendment is proper, inasmuch as Applicants could not previously have provided the amendments, inasmuch as they are responsive to rejections which are newly made in the Final Rejection.

**Sequence Rules Compliance and Objection to claim 61**

It is submitted that the discussion of the sequence rules compliance, and the objection to claim 61, is rendered moot by cancellation of this claim. Withdrawal of these issues is respectfully requested.

**Rejection Under 35 U.S.C. 102**

Claims 42-44, 46, 47, 51-56, 59-60 and 64 have been rejected under 35 U.S.C. 102(b) over Tyagi '517. Reconsideration of this rejection is respectfully requested.

Tyagi fails to disclose a fluorescent conjugate comprising a fluorescent entity which is a cyanine dye. Instead, Tyagi discloses "hybridization probes" employing:

- a single stranded nucleic acid sequence that is complementary to a desired target nucleic acid (the "target complement sequence")
- 5' and 3' regions flanking the target complement sequence that reversibly interact by means of either a complementary nucleic acid sequences or by attached members of another affinity pair, and
- interactive label moieties for generating a signal. See col. 4, line 62 – col. 5, line 2.

Patentees' hybridization probes can be either unimolecular or bimolecular, see col. 6,

lines 6-12. A bimolecular version of these probes would therefore be constituted of:

- a single stranded nucleic acid sequence,
- a flanking region which can be a member of an affinity pair, such as an antibody, and
- a label moiety.

The probe according to U.S. '517, whether unimolecular or bimolecular, comprises interactive label moieties (such as fluorescent FRET partners) which proximity to each other is regulated by the status of the interaction of the affinity pair.

When the target complement sequence hybridizes to its target, a conformational change occurs in the probe, separating the affinity pair and consequently the label moieties. Upon separation, a change in the signal resulting from the label moieties can be observed.

Although Tyagi briefly mentions (col. 9 1.40 and claim 43) the possibility for the "affinity pair" to comprise an antibody; patentees teach that the use of complementary nucleic acid sequences as the affinity pair is preferred since they are technically very easy to conjugate to the "target complement sequence" and the parameters governing their interaction/binding are the same as the ones governing the hybridization of the target complement sequence to its target.

Accordingly, Tyagi, which fails to disclose a cyanine dye, further falls far short of disclosing a cyanine dye covalently attached to an oligonucleotide, and a carrier molecule which is an antibody or a protein, covalently attached by a functional group on the cyanine dye or the oligonucleotide.

It is moreover respectfully submitted that, not only does Tyagi fail to disclose the present claims, but also fails to suggest them. As noted above, Tyagi is directed to the production of hybridization probes. Patentees do not address, or propose a solution, to the problem of cyanine dye aggregation (not surprising, since cyanine dyes are not disclosed). Surprisingly, the present inventors have discovered that oligonucleotides can prevent aggregation of organic dyes, (more particularly cyanine dyes), which is usually observed when they are conjugated to proteins (such as antibodies), and thus the invention avoids a drastic decrease of fluorescence efficiency (reduction in quantum yield).

This surprising result is demonstrated in example 8 of the present application.

In this example, the quantum yield of a cyanine (Cy5, sulfonated or unsulfonated) was measured when unconjugated or conjugated with an antibody (GSS11); the results are presented in tables 1 and 2 of the specification, which are reproduced here for convenience:

Table 1

| | Final molar ratio (FMR) | $OD_{maz}/OD_{604\ nm}$ | Quantum yield |
|---|---|---|---|
| CY5sulfo-mono NHS | ---------- | 3.16 | 19% |
| GSS11-CY5sulfo batch M5 | 2.20 | 2.18 | 11% |
| GSS11-CY5sulfo batch M6 | 3.80 | 1.64 | 4% |
| GSS11-CY5sulfo batch M7 | 6.00 | 1.24 | 1% |

Table 2

| | Final molar ratio (FMR) | $OD_{maz}/OD_{604\ nm}$ | Quantum yield |
|---|---|---|---|
| CY5-T15-hexylamine | ---------- | 2.79 | 25% |
| GSS11-T15-CY5 batch 02B (mal) | 4.30 | 2.59 | 19% |
| GSS11-T15-CY5 batch 03 (mal) | 1.40 | 2.59 | 18% |
| GSS11-T15-CY5 batch 01 (NHS) | 2.00 | 2.67 | 20% |
| GS11-T15-CY5 batch 02 (NHS) | 5.10 | 2.63 | 20% |
| CY5-T10-hexylamine | ---------- | 2.78 | 26% |
| GSS11-T10-CY5 batch 01 (mal) | 4.60 | 2.38 | 14% |

Sulfonation of cyanines is known to reduce aggregation of hydrophobic cyanines in solution, Sulfonated cyanines are, however, still subject to aggregation when conjugated to an antibody, as shown in table 1.

When the final molar ratio increases, i.e., when several cyanines are conjugated per antibody, the quantum yield is of 19%, but as soon as it is conjugated to antibody GSS11, it

drops to 11% when an average of 2.2 cyanines are conjugated per antibody and to 4% when an average of 3.8 cyanines are conjugated per antibody.

On the other hand, as shown in table 2, when a Cy5-oligunucleotide conjugate is used instead of a cyanine, quantum yield is preserved after coupling with the antibody GSS11, even when as many as 5.1 cyanines are conjugated per antibody.

The cited references do not suggest that cyanines substituted by an oligunucleotide would be resistant to aggregation when used to label a protein, and particularly an antibody.

Thus, there is absolutely no motivation to synthesize cyanine dyes substituted by an oligonucleotide and further conjugated to a protein: cyanine dyes have been known for decades as fluorescent labels and the used to manufacture conjugates of cyanine dyes with *either* nucleotides, oligonucleotides, proteins or other bimolecules, but not to synthesize the conjugates such as those claimed herein.

Because fluorescent labeling of oligonucleotide is typically performed only in order to study genes or DNA structure or sequences, one of ordinary skill in the art trying to find a way to prevent aggregation of cyanine dye on the surface of proteins, in a context that has nothing to do with DNA study would *NOT* be motivated to go through the burden of conjugating a cyanine dye with both an oligonucleotide and a protein, particularly in view of the unexpected results detailed above.

Accordingly, it is respectfully submitted that, not only does Tyagi fail to disclose the presently claimed invention, it further fails to suggest it. Withdrawal of the rejection is therefore respectfully requested.

**Rejection Under 35 U.S.C. 103**

Claims 45, 48, 57 and 62 have been rejected under 35 U.S.C. 103 over Tyagi taken with Glazer. Moreover, claims 49 and 58 have been rejected under 35 U.S.C. 103 over Tyagi and Glazer taken with Surrey. Reconsideration of each of these rejections is respectfully requested.

As noted above, Tyagi fails to disclose the conjugates presently claimed. Glazer is cited for teachings of in particular linear alkylene spacer arms. Surrey is cited for a disclosure of alkyldiamides. Thus, neither one addresses the deficiencies discussed above, and it is submitted

that, for the reasons discussed above, these rejections should also be withdrawn.

The claims of the application are submitted to be in condition for allowance. However, if the Examiner has any questions or comments, he is cordially invited to telephone the undersigned at the number below.

The Commissioner is hereby authorized to charge any fees associated with this response or credit any overpayment to Deposit Account No. 13-3402.

Respectfully submitted,

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